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THE CHROMOSOME CYCLE IN NEMATODES*
A PRELIMINARY REPORT

By
A. C. Walton

During the course of the past nine years the writer has had opportunities of making intensive studies of the formation of the germinal elements of a considerable number of the round worms which are parasitic in some of our well-known birds and mammals. During the past year while working at the University of Illinois, a comparative study was made of the material of sixteen different forms and some rather definite and interesting generalizations were found, as well as the specific course of events being deter-

Table I

Super-family	Family	Genus	Species	Host
Ascaroidea	*Ascaridae (Ascarinae)*	*Ascaris*	*lumbricoides*	Pig
			megalocephala biv.	Horse
			megalocephala univ.	Horse
		Belascaris	*mystax*	Cat
			triquetra	Dog
		Toxascaris	*canis*	Dog
	Ascaridae (Anisakinae)	*Contracaecum*	*spiculigerum*	Pelican
	Heterakidae	*Ganguleterakis*	*spumosa*	Rat
		Heterakis	*papillosa*	Chicken
Filaroidea	*Acuariidae (Acuariinae)*	*Acuaria*	*spiralis*	Grackle
	Acuariidae (Physalopterinae)	*Physaloptera*	*turgida*	Opossum
	Spiruridae	*Protospirura*	*muris*	Rat
Oxyuroidea	*Cruzidae*	*Cruzia*	*tentaculata*	Opossum
	Oxyuridae	*Syphacia*	*obvelata*	Rat
Strongyloidea	*Trichostrongylidae*	*Nematospira*	*turgida*	Mouse
Trichinelloidea	*Trichinellidae*	*Trichosomoides*	*crassicauda*	Rat

*Contribution from the Zoological Laboratory of the University of Illinois, under the direction of Henry B. Ward, No. 237.

mined for each form examined. I wish here to express my appreciation to Dr. H. B. Ward, Director of the Laboratories, for his assistance in the carrying out of this work and in affording me the privileges of the laboratory.

The sixteen species studied represented twelve genera, ten sub-families, eight families and five super-families of the order Nematoda. The preceding table gives the latest classification of the parasites and also gives the host from which the material in question was obtained.

In one case a new genus and species—*Nematospira turgida*—were established. *Acuaria spiralis*, *Cruzia tentaculata* and *Syphacia obvelata* were found in new hosts and several forms were reported from new localities. The material of *Belascaris triquetra* used was taken from a dog, the usual host animals for this parasite being lions, wolves and bears. The dog, however, has long been known to be an occasional host to this form.

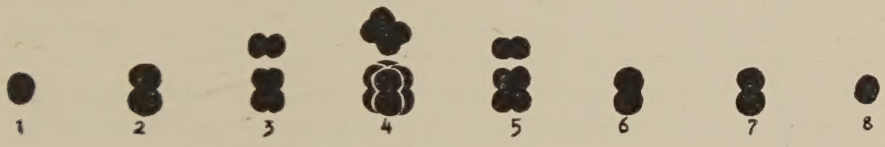
For the sake of clearness, a few definitions of technical terms used in describing the germ cycle may well be included at this place.

gametogenesis	=the process of gamete or sex-cell development.
oogenesis	=the process of female sex-cell development.
spermatogenesis	=the process of male sex-cell development.
maturation	=the maturing of the germ cells.
mitosis	=the process of ordinary cell division accompanied by chromosome formation.
meiosis	=refers to the two maturation mitoses only.
oogonia	=female germ cells in early stage of development.
spermatogonia	=male germ cells in early stage of development.
haploid	=number of chromosomes in mature germ cells.
diploid	=number of chromosomes in fertilized egg and in immature germ-cells and occasionally in body cells.
tetraploid	=number of chromosomes often found in the somatic cells and occasionally in the primordial germ cells.
monad	=simplest morphological form of the chromosome.
dyad	=chromosome equivalent to two monads.
tetrad	=chromosome equivalent to four monads.
di-tetrad	=chromosome equivalent to eight monads.
autosome	=chromosome bearing no relation to sex.
heterochromosome	=chromosome bearing a definite relation to sex.
idiochromosome	=synonymous with heterochromosome.
diminution	=a process occurring during the somatic cell divisions of the early embryo in which a certain portion of the chromatin is extruded into the cytoplasm and the rest retained within the nucleus. This process occurs only once in any one cell.
interkinesis	=interval between the active phases of two successive cell divisions. Often restricted to the period between the two maturation divisions.
querkerbe	=term applied to the surface indication of the plane of union of two monads to form a dyad.

During the progress of this study it was noted that a large number of Nematodes showed the simplest morphological form of their chromosomes

in the body cells of the embryo, and that in the majority of cases these bodies were twice as numerous as those of the germ cells of the embryo. In other words, the somatic chromosomes, and occasionally the primordial germ cell chromosomes, are tetraploid in number and simple in structure while the germ-cell chromosomes are diploid in number and complex (at least two-parted, a dyad) in structure. The same relation is found in the matured egg and spermatozoon as regards the structure, but the number of chromosomes is halved, i.e., it is the diploid number. Just before the second maturation division the chromosomes are haploid in number and complex—tetrad—in structure. In such cases they are haploid in number and di-tetrad in structure before the first maturation division begins. These di-tetrads are formed by the pairing of a paternally and a maternally derived tetrad, and each tetrad had been formed by a longitudinal splitting of a preceding dyad. These tetrads and dyads are present in diploid number which is changed to the haploid by the process of pairing. This process is usually spoken of as *pseudo-reduction* since there is probably no actual intermingling of the contents of the members of a pair, but only a temporary apposition followed by a complete separation along the plane of union at the time of the qualitative, or reducing, division.

Textfigure A diagrammatically shows a complete cycle of a chromosome from a simple somatic form to a complex maturing germ-cell structure.



TEXTFIGURE A

Figure A. Complete cycle of a di-tetrad form of Nematode chromosome.
1. Monad form, found in tetraploid number in soma cells and primordial germ cells.
2. Dyad form, found in diploid number in gonial cells. Formed by a fusion of two monads.
3. Tetrad form, found in diploid number in Prophase I. Is a longitudinally split dyad.
4. Di-tetrad form, found in haploid number in Metaphase II. Formed by pairing of tetrads.
5. Tetrad form, found in haploid number in Prophase II. Formed by splitting of di-tetrad.
6. Dyad form, found in haploid number in mature germ cells. Formed by splitting of tetrad.
7. Dyad form, found in diploid number in fertilized eggs. 8. Monad form, same as in 1.

In valances, the cycle is expressed in the following manner (the succession is the same as that in the text figure):

1	2	4	8	4	2	2	1
monads	dyads	tetrads	di-tetrads	tetrads	dyads	dyads	monads

The valance of the somatic monad chromosomes is assumed to be *one*.

With but very few exceptions, the usual chromosome cycle has shown a tetrad with the valance of *4* as the most complex member. These exceptions have been regarded as accidental or as fragmentation processes

and not of consistant presence or meaning. This study has shown that a great majority of the nematodes examined possess a di-tetrad form of chromosome, and in a few cases a much more complex type, but always one which is constant within a species.

The following is a brief summary of the results gained by a study of sixteen species. The members of the *Ascarinae* are discribed first because of their apparently greater specialization and further removal from the primitive Nematode conditions.

(1) *Ascaris lumbricoides.* The haploid number consists of twenty-four chromosomes in the females. One-half of the males possess nineteen tetrad autosomes, the other half nineteen tetrad autosomes and five dyad heterochromosomes. The hetero-chromosome complex of five members is of the X type. The diploid number is forty-three in the males and forty-eight in the females, all monads in structure. One of the heterochromosome members is consistently larger than its mates. The diploid chromosomes are each physiologically equivalent to three bodies, two of which are lost at diminution and the third remains as the somatic chromosome. The heterochromosome complex does not form a separate karyosome during interkinesis in either spermatogenesis or oogenesis.

(2) *Ascaris megalocephala bivalens.* The germ cell chromosomes were determined to be each equivalent to twenty-two somatic elements. The attached X chromosome is equivalent to eight somatic bodies. The somatic number of chromosomes is ninety-six or one hundred and four, depending upon the sex of the worm studied.

(3) *Ascaris megalocephala univalens.* The germ cell chromosomes were determined to be each equivalent to twenty-six somatic chromosomes. The attached X chromosome is equivalent to eight somatic bodies. The somatic number of chromosomes is fifty-two or sixty depending upon the sex of the worm studied.

(4) *Belascaris mystax.* The haploid number consists of nine chromosomes in each sex. Eight of these are tetrads and one a hexad—tetrad autosome plus dyad heterochromosome. The single heterochromosome is of the X type and is normally attached to the end of an autosome. The single heterochromosome is of the X type and is normally attached to the end of an autosome. The diploid number of chromosomes is eighteen, all monads. The diploid chromosomes are morphologically simple, the somatic cells showing the same number and structure of the chromosomes as do the germ cells. The diminution process occurs at the first division of each soma cell after its separation from the propagation cells. The idiochromosome forms a separate karyosome during the interkinetic periods of spermatogenesis.

(5) *Belascaris triquetra.* The haploid number consists of twelve ditetrad chromosomes in the female. One-half of the males possess ten di-

tetrad autosomes and the other half ten di-tetrad autosomes and two tetrad heterochromosomes. The heterochromosome complex consists of two X bodies. The diploid number of chromosomes is twenty-two for the males and twenty-four for the females, all dyad in structure. The diploid chromosomes are each equivalent to two somatic elements as well as to a small amount of chromatin which is lost by a diminution process. The somatic numbers of chromosomes are forty-four and forty-eight, according to the sex of the worms studied. The heterochromosomes are isolated from the autosomes during both spermatogenesis and oogenesis by the formation of separate karyosomes for each type of chromosome.

(6) *Toxascaris canis*. The haploid number consists of eighteen di-tetrad chromosomes in the females. One-half of the males have twelve di-tetrad autosomes and the other half have twelve di-tetrad autosomes and six tetrad heterochromosomes. The latter form a heterochromosome complex of the X type. The diploid number of chromosomes is thirty in the males and thirty-six in the females, all of them dyads. The diploid chromosomes are duplex, each being equivalent to two somatic chromosomes, and in addition to a small amount of chromatin lost at diminution. The somatic number of chromosomes is sixty and seventy-two, all being monad in structure. The heterochromosomes form independent karyosomes during interkinetic periods of the male germ cycle.

(7) *Contracaecum spiculigerum*. The haploid number consists of five di-tetrad chromosomes in the female. One-half of the males possess four di-tetrad autosomes and the other half four di-tetrad autosomes and a tetrad heterochromosome of the X type. The diploid number of chromosomes is nine in the males and ten in the females, all dyads in structure. The diploid chromosomes are each equivalent to two somatic chromosomes. These germ cell chromosomes also reduce to a tetraploid number in the primordial sex cells. The tetraploid number is eighteen and twenty, all monads in structure. A diminution division does not occur. The heterochromosome is not isolated from the autosomes during interkinesis by the establishment of a separate karyosome.

(8) *Ganguleterakis spumosa*. The haploid number consists of six tetrad-like chromosomes in the female. One-half of the males possess four tetrad autosomes, and the other half possess four tetrad autosomes and two tetrad heterochromosomes of the X type. The autosomes are potentially di-tetrads in that they again show a tetrad formation after the first maturation division is completed. The diploid number of chromosomes is ten in the males and twelve in the females, all dyad in structure. The diploid chromosomes are each equivalent to two somatic chromosomes. A diminution process does not occur. The primordial germ cells as well as the somatic cells show a tetraploid number of twenty and twenty-four monad chromosomes. The heterochromosomes are not isolated from the autosomes by

the establishment of separate karyosomes during either spermatogenesis or oogenesis.

(9) *Heterakis papillosa.* The haploid number consists of five di-tetrad chromosomes in the female. One-half of the males possess four di-tetrad autosomes and the other half four di-tetrad autosomes and a tetrad heterochromosome of the X type. The diploid number of chromosomes is nine in the males and ten in the females, all dyads in structure. The diploid chromosomes are each equivalent to two somatic chromosomes. The germ cell chromosomes also reduce to a tetraploid number in the primordial sex cells. This tetraploid number is eighteen or twenty according to the sex, and each chromosome is monad in form. A diminution process was not observed. The heterochromosome is isolated from the autosomes during the interkinetic periods of spermatogenesis by the establishment of a separate karyosome.

(10) *Acuaria spiralis.* The haploid number consists of six tetrad-like chromosomes for the females. One-half of the males have five tetrad-like autosomes and the other half have six such bodies, one of them being a tetrad heterochromosome of the X type. These autosomes are physiologically di-tetrads, but the second longitudinal split fails to become manifest until after the first division has been completed. The diploid number is eleven for the males and twelve for the females. The diploid chromosomes are each equivalent to two somatic chromosomes. The primordial germ cells as well as the somatic cells have a tendency to show the tetraploid numbers of twenty-two and twenty-four monad chromosomes. A diminution process is not present during somatic mitoses. The heterochromosome forms an individual karyosome during the interkinetic periods of spermatogenesis but not of oogenesis.

(11) *Physaloptera turgida.* The haploid number consists of five tetrad chromosomes in the females. One-half of the males show four tetrad autosomes, and the other half four tetrad autosomes and a tetrad heterochromosome of the X type. The autosomes again appear as tetrads before the second maturation division by the formation of a second longitudinal cleft. The diploid number of chromosomes is nine for the males and ten for the females, all dyads in structure. The diploid chromosomes are each physiologically equivalent to two somatic chromosomes although the soma cells only rarely show the tetraploid number of eighteen or twenty monads. No traces of a diminution phenomenon were observed. The heterochromosome not only does not form a separate karyosome in resting nuclei, but is so closely related to an autosome that the latter is much delayed in division, both in spermatogenesis and in oogenseis.

(12) *Protospirura muris.* The haploid number consists of five di-tetrad chromosomes in the females. One-half of the males possess four di-tetrad

autosomes and the other half possess four di-tetrad autosomes and a tetrad heterochromosome of the X type. The diploid number of chromosomes is nine for the males and ten for the females. The diploid chromosomes are each equivalent to one somatic chromosome. Neither the somatic cells nor the primordial germ cells have any tendency to show a tetraploid number of monad chromosomes, but retain their dyad structure and diploid number throughout the life cycle. There is no evidence of a diminution process and no tendency for the heterochromosome to form a separate karyosome at any time.

(13) *Cruzia tentaculata*. The haploid number of chromosomes is six for the females. One-half of the males have five di-tetrad autosomes, and the other half have five di-tetrad autosomes and a tetrad heterochromosome of the X type. The diploid chromosomes are equivalent to two somatic chromosomes. Diminution probably does not occur. The primordial germ cells as well as the somatic cells show the tetraploid number of twenty-two or twenty-four monad chromosomes. The heterochromosome is not isolated from the autosomes by the formation of a separate karyosome during the interkinetic periods of spermatogenesis.

(14) *Syphacia obvelata*. The haploid number consists of eight tetrad chromosomes in the female. One-half of the males show seven tetrad autosomes, and the other half show seven tetrad autosomes and one dyad heterochromosome of the X type. The diploid number is fifteen for the males and sixteen for the females, all monads in structure. The germ cell and the somatic cell chromosomes are morphologically equivalent. There is no isolation of the heterochromosome at any period through the formation of separate karyosomes and there is no evidence of any diminution process.

(15) *Nematospira turgida*. The haploid number consists of six di-tetrad chromosomes in the females. One-half of the males show five di-tetrad autosomes and the other half five di-tetrad autosomes and a single heterochromosome of the X type. The diploid number is eleven for the males and twelve for the females, all dyads in structure. The diploid chromosomes are morphologically equivalent to two somatic chromosomes although the soma cells but rarely show a reduction to the tetraploid number of monad chromosomes. No traces of a diminution process were observed. The idiochromosome maintains its distinct form during meiosis, but apparently fuses with the other chromatic material during the balance of the germ cycle.

(16) *Trichosomoides crassicauda*. The haploid number consists of four di-tetrad chromosomes in the female. One-half of the males show three di-tetrad autosomes and the other half show three di-tetrad autosomes and a single tetrad heterochromosome of the X type. The diploid number of

chromosomes is seven for the males and eight for the females, all dyads in structure. The somatic cell chromosomes as well as those of the germ cells retain their dyad structure throughout the entire life cycle, no evidence of a reduction to a monad condition being found at any stage. Diminution also fails to occur. The heterochromosome does not remain isolated in a separate karyosome during the resting stages of any of the cells. The chromosomes are definitely 'V' shaped throughout the germ cycle except during the maturation period. Here they assume the typical di-tetrad form of many of the Nematodes, and reduce through tetrads to dyads before they resume the 'V' shape again. The spermatozoa are peculiar in possessing a whip-like tail as well as an amoeboid head. There is no evidence of glycogen storage at any period of the spermatozoon metamorphosis.

The table on page 9 sums up the results of the foregoing studies.

Examination of the results of the above studies and of the work of other investigators has led to the formation of some general conclusions as regards to *processes*:—

(1) The presence of di-tetrad haploid chromosomes is an indication that the germ cell chromosomes are compound, each being equivalent to two monad chromosomes such as are usually found in somatic cell nuclei and occasionally in the primordial germ cell nuclei. The Querkerbe of the germ cell chromosomes indicates the point of this union, which may or may not be permanent. When permanent, the chromosomes retain their dyad condition throughout the germ cycle.

(2) The compound nature of the meiotic chromosomes is well illustrated by a series beginning with *Syphacia obvelata* in which the diploid chromosomes are monads like those of the soma cells. A second stage is found in *Belascaris mystax* and *Acuaria spiralis* where the germ cell chromosomes are equivalent to one somatic chromosome and, in addition, to some chromatin which is lost by diminution. *Ascaris lumbricoides*, in which a germ cell chromosome is equivalent to one somatic chromosome plus two definite bodies lost at diminution, is also in this group of the series. Next comes a number of species in which the haploid chromosomes are di-tetrads, reducing to dyad diploid bodies, each of which is equivalent—either physiologically or morphologically—to two somatic chromosomes. *Toxascaris canis* is an example of this group. The series culminates in such forms as the varieties of *Ascaris megalocephala* in which each germ-cell chromosome is equivalent to twenty-two or twenty-six somatic chromosomes, and the heterochromosome to eight. In addition each autosome loses some excess chromatic material at the diminution division.

(3) The presence of the tetraploid—or even higher—number of chromosomes in the soma cells of Nematodes is not an indication of a fragmentation process, but rather indicates the phylogenetically simple number for the

TABLE II

Name	Haploid number of chromosomes	Diploid number of chromosomes	Diminution phenomenon	Somatic number of chromosomes	Heterochromosome number	Autosome unit value
Acuaria spiralis	6 (di-tetrads)	11-12 (dyads)	undetermined	22-24	X=1	2
Ascaris lumbricoides	24 (tetrads)	43-48 (monads)	present	43-48	X=5	1+2
Ascaris megalocephala biv.	2 (rod-shaped)	4 (rod-shaped)	present	96-104	*X=1	22+
Ascaris megalocephala univ.	1 (rod-shaped)	2 (rod-shaped)	present	52-60	*X=1	26+
Belascaris mystax	9 (tetrads)	18 (monads)	present	18	X=1	1+
Belascaris triquetra	12 (di-tetrads)	22-24 (dyads)	present	44-48	X=2	2+
Contracaecum spiculigerum	5 (di-tetrads)	9-10 (dyads)	absent	18-20	X=1	2
Cruzia tentaculata	6 (di-tetrads)	11-12 (dyads)	undetermined	22-24	X=1	2
Ganguleterakis spumosa	6 (di-tetrads)	11-12 (dyads)	absent	20-24	X=2	2
Heterakis papillosa	5 (di-tetrads)	9-10 (dyads)	undetermined	18-20	X=1	2
Nematospira turgida	6 (di-tetrads)	11-12 (dyads)	absent	22-24	X=1	2
Physaloptera turgida	5 (tetrads)	9-10 (dyads)	absent	18-20	X=1	2
Protospirura muris	5 (di-tetrads)	9-10 (dyads)	absent	9-10	X=1	>+1
Syphacia obvelata	8 (tetrads)	15-16 (monads)	absent	15-16	X=1	1
Toxascaris canis	18 (di-tetrads)	30-36 (dyads)	present	60-72	X=6	2+
Trichosomoides crassicauda	4 (di-tetrads)	7-8 (dyads)	absent	>++7-8	X=1	2

>* Heterochromosome unit value equals eight.

>+ Somatic chromosomes are dyad in structure.

>++ Somatic chromosomes and primordial germ cell chromosomes are 'V' shaped.

species and points to the fact that the germ-cell chromosomes are compounds of a definite number of these simple somatic chromosomes; the Querkerbe, when present, indicating the plane of such fusion. The presence of the tetraploid number of chromosomes in both the somatic cells and in the primordial germ cells points to another line of evidence as to the simplicity or complexity of the species from a phylogenetical standpoint.

(4) All of the Nematodes studied show a heterochromosome complex of the X type, usually one in number, but consisting of two members in *Belascaris triquetra* and *Ganguleterakis spumosa*, five in *Ascaris lumbricoides* and six in *Toxascaris canis*.

(5) The relationship of the heterochromosomes to the autosomes is illustrated by a series in which the heterochromosomes are formed from a separate karyosome in both the males and the females—*Belascaris triquetra*,—from a separate karyosome in males only—*Toxascaris canis*,—are distinct from the autosomes only during the maturation division period—*Cruzia tentaculata*,—are physiologically associated with an autosome—*Physaloptera turgida*,—are visibly connected to an autosome—*Belascaris mystax*,—and finally, are attached to an autosome so closely that their presence cannot be detected except in isolated instances—*Ascaris megalocephala*.

(6) There is no wide-spread tendency for the isolation of heterochromosome from autosome material during interkinesis.

(7) Diminution is found in all of the *Ascarinae* studied but absent in all the others, apparently being restricted to members of this one sub-family. Goodrich ('16) reports the presence of an 'X-Y' group of very great complexity and also the presence of a diminution division in a form from the shark, *Ascaris incurva*. This form has recently been reclassified as a *Contracaecum*, and as a member of this genus, gives an example of another sub-family of the *Ascaridae*, the *Anisakinae*, to show the phenomenon. Until further study of this form can be made, both cytologically and morphologically, its importance and its position with reference to the general statements given above can not be estimated.

A more detailed report on the material presented above will appear at an early date in the Zeitschrift für wissenschaftliche Biologie.

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OBSERVATIONS ON THE HABITS AND LIFE HISTORY OF GALLERUCELLA NYMPHAEAE (COLEOPTERA)[1]

By

Helen M. Scott

During the summers of 1918 and 1923 the writer carried on a study of *Gallerucella nymphaeae* (L.) at the University of Michigan Biological Station on Douglas Lake, Michigan, with the hope of securing data which would extend knowledge of the habits and life history of this insect. This beetle and its principal food plant, the yellow waterlily (*Nymphaea advena* Ait.),[2] are very abundant in certain regions of Douglas Lake and in the adjacent creeks and pools and throughout the whole summer material for study was always available. Results obtained are confirmatory of certain data presented by previous workers, modify and amplify certain earlier work, and add entirely new information concerning this form. The life history stages as manifested in the summer months, was worked out completely and all of the instars determined. However, detailed descriptions of the larval instars will be reserved for a later paper.

The writer is indebted to Dr. Paul S. Welch under whose direction the work was done, and to Drs. H. A. Gleason, J. H. Ehlers, and F. C. Gates for assistance in the identification of the food plants.

Eggs

The eggs occur in masses, arranged in often closely set rows on the upper surface of the leaves of *Nymphaea advena* and *Polygonum hydropiperoides*. One hundred and twenty-six egg masses, collected in the field, were studied and the number of eggs in each found to vary from two to nineteen, with an average of eleven. McGillivray (1903, p. 325) reported a variation of six to twenty eggs per cluster. Measurements of fourteen eggs, selected from different masses showed the following dimensions: length, 0.57 to 0.70 mm., average, 0.63 mm.; thickness, 0.4 to 0.60 mm., average 0.49 mm. Weiss and West (1920, p. 237) reported the length of the egg as 0.9 mm. and the width as 0.5 mm. The eggs are oblong with ends rounded; however, the end attached to the leaf is the broader and is slightly flattened. The chorion has hexagonal, depressed surface areas in which minute marking may be detected under high magnification. At the smaller, rounded end of the egg the depressed areas become irregularly arranged about a smooth portion of the chorion. When first deposited, the color of the egg is a deep

[1] Contribution from the University of Michigan Biological Station.

[2] According to Miller and Standley (1912, pp. 78-82) this species should be designated as *Nymphaea americana* (Provancher).

yellow but after a few minutes it changes to an ivory appearance. The contents of the egg are yellow with a very small quantity of clear white material around the yolk.

On contact with water the egg becomes wetted. Since wetting and even temporary submergence of the egg masses is an occasional, normal occurrence in nature, experiments were performed to try to determine the effects of submergence but conclusive results were lacking since in one series larvae emerged from the submerged eggs and crawled up to a leaf above water, while in another series submergence for two weeks yielded no larvae.

Larva

Since a description of the larva is given by McGillivray (1903, pp. 325-326) none will be included here. In the hatching process the chorion is cracked, after which the larva within the shell breaks off the edges, bit by bit, by means of its mouth parts, until the opening is large enough to crawl through. The aperture thus formed is often a little to one side of the middle of the egg. Emergence is usually a slow process. The larva begins feeding immediately after freeing itself from the egg shell.

Instars

In the breeding work egg masses were brought into the laboratory directly from the field in order to obtain and record the larvae as soon as they were hatched. In each case, some of the larvae of the first instar were killed and preserved for future anatomical study, while others were placed in breeding jars, on the leaves of the yellow waterlily, and observed at frequent intervals until the first larval molt. The exuviae remain on the leaf thus facilitating the detection of molting. After the first larval molt, a certain number of the larvae of the new instar were killed and preserved, while others were isolated in breeding jars on fresh food plant leaves to await the next molt. This method was employed until six individuals had been brought through to the imago stage.

First Instar.—The length of the first larval period varied from three to five days. Sixteen larvae in the first instar were measured and found to possess the following dimensions: length, 1.33—1.61 mm., average, 1.55 mm.; maximum body width 0.38—0.47 mm., average 0.42 mm.; head capsule, 0.19—0.28 mm., average 0.19 mm.

Second Instar.—The length of the second larval instar varied from two to seven days. Measurements of nine larvae showed the following dimensions: length 2.8-3.99 mm., average 3.37 mm.; maximum body width 0.76-1.1 mm., average 0.94 mm.; head capsule 0.33-0.47 mm., average 0.36 mm.

Third Instar.—The length of the third larval instar varied from two to seven days. Examinations of eleven larvae showed the following dimen-

sions: length 3.89-6.55 mm., average 4.87 mm.; maximum body width 1.23-1.71 mm., average 1.47 mm.; head capsule 0.47-0.66., average 0.5 mm.

Larval Period

Six larvae, distributed among four different breeding jars, were carried through from the hatching time to pupation in from seven to nineteen days. The variation in time seems considerable especially since the conditions of rearing were essentially similar. All rearing work was carried on in the laboratory and while no breeding experiments were performed entirely in the field for comparison, there appeared to be nothing in the indoor work which imposed any serious abnormal conditions.

Food Plants and Food Habits

The plants on which the larvae have been found feeding are *Nymphaea advena*, *Polygonum hydropiperiodes*, *Polygonum amphibium*, and *Myrica gale*. The last two mentioned plants will be discussed in connection with the experiments with the larvae. The larvae crawl about at random over the upper surface of the emergent leaves, making irregular traces as they eat off the upper parts of the leaf tissue. Fresh feeding grooves are easily detected since for a short time after they are produced they are green in appearance; exposure to the air, however, soon causes these feeding areas to turn brown. Night observations made by means of flash lights showed that the larvae feed actively at night as well as during the day. Whether the total food consumption at night was as great as that made during the day was not determined.

Immersion Experiments

Experiments showed that the larvae are resistant to a certain amount of submergence in water. This exigency frequently happens in their normal environment, although obviously long continued submergence would not be possible in larvae so characteristically terrestrial in structural makeup. Larvae when submerged have a distinct silvery appearance, showing that an air film surrounds the body. Irregular movements of the abdomen appeared and seemed to represent the only response of the larvae to short periods of immersion. Submergence periods of as long as ten minutes did not result in change of position. Effects of long submergence were not studied.

Food Experiments

In the natural habitat other plants, notably *Castalia odorata* and *Potamogeton natans*, intermingle closely with *Nymphaea advena* and experiments were performed to determine if they also might serve as food plants. Larvae of various sizes and ages were introduced into four

aquaria containing *Castalia odorata* leaves only and into four aquaria containing *Potamogeton natans* leaves only. In no case were the leaves of either plant eaten.

Previous investigators (Chittenden, 1905; Weiss and West, 1920) mention reports of larvae feeding on *Sagittaria*, *Brasenia*, and *Nuphar*. Of these plants, only Sagittaria occurs about Douglas Lake. Leaves of *Sagittaria latifolia* were brought into the laboratory, placed in aquaria, and larvae of various sizes and ages introduced. After an exposure of one week no evidences of feeding were observed.

Pupae were found in the field on the leaves of *Myrica gale* and signs of the eating by either the adult or the larva were evident. A branch of *Myrica gale* was brought into the laboratory, the end placed in water, and larvae of various ages and sizes were transferred to the leaves. The larvae ate the leaves readily and the experiment was continued until the larger ones had pupated.

In an aquarium, the leaves of *Polygonum amphibium* were readily eaten by the larvae.

Habits of the Larvae

On several occasions larvae are observed feeding upon the egg masses of their own species. The eggs were broken by the mouth parts and the contents eaten. When a larva had finished with an egg mass only the part of the chorion attached to the supporting leaf remained. Evidently the larvae constitute an enemy to their own species.

Pupa

The pupal period varied from five to six days. Of six larvae carried through to the adult stage, five required six days and one five days as pupae.

Imago

Reproductive Capacity

In order to secure some information concerning the egg-laying capacity of the females, beetles found mating in the field were brought into the laboratory and placed in aquaria, each pair to itself, on leaves of *Nymphaea advena*. The following results were obtained: *Pair no. 1:* July 19, 10 eggs; July 21, 9 eggs; July 22, 7 eggs; July 25, 8 eggs; July 26, 6 eggs; July 27 6 eggs; July 28, 6 eggs; July 29, 11 eggs; July 30, 10 eggs; July 31, 11 eggs; Aug. 1, 10 eggs; Aug. 3, 10 eggs; Aug. 6, 11 eggs; *total* over period of nineteen days 115 eggs.– *Pair no. 2:* July 26 to Aug. 1 inclusive, 9 eggs per each succeeding day; Aug. 6, 9 eggs; Aug. 14, 7 eggs; Total over period of eighteen days 79 eggs. *Pair no. 3.* July 28, 12 eggs; Aug. 2, 12 eggs; Aug. 6, 12 eggs; *total* over period of ten days.

Another record, consisting of the use of one female taken immediately after mating in the field and continuously isolated in an aquarium with plenty of food plant, is as follows: Aug. 3, 10 eggs; Aug. 4, 10 eggs; Aug. 6, 10 eggs; Aug. 8, 10 eggs; Aug. 9, 10 eggs; Aug. 13, 8 eggs; *total* over period of ten days 58 eggs.

These results are not offered as an exact indication of the complete egg laying capacity since it was realized that in the selection of mating females in nature there was no way of knowing whether matings previous to the one observed had occurred. They do, however, show something as to the rate of oviposition, the period over which oviposition may occur, and possible totals which may be reached and even exceeded in the production of eggs.

Oviposition

In only one case was a female observed throughout the whole process of oviposition. It required one hour and twenty eight minutes to deposit one egg mass containing eleven eggs. However, it is evident that the rate of oviposition is highly variable since observations on egg laying in the field showed that a female in a state of active oviposition may deposit as many as eleven eggs in eight minutes.

Previous to the deposition of an egg, the female moves the tip of the abdomen back and forth over a small area of the leaf surface, the effect of which seems to be visible, when an egg mass is removed, in the form of tiny punctures on the surface of the epidermis.

Food Plants

In the field, adults were found feeding on *Nymphaea advena* and *Polygonum hydropiperoides*. *Myrica gale* and *Polygonum amphibium* showed signs of having been eaten by this beetle and were therefore brought into the laboratory and introduced into aquaria together with the adult beetles. The leaves of both plants were readily eaten. According to the field observations the leaves, petioles, petals, sepals, and the flower buds of *Nymphaea advena* are attacked by this beetle but of the other food plants only the leaves are consumed. Late in summer, in the environs of Douglas Lake, it was almost impossible to find leaves of *Nymphaea advena* which had not been attacked by the larvae and adults. Feeding activity, if of any considerable amount, gives to the leaves of both *Nymphaea advena* and *Polygonum hydropiperoides* a dilapidated appearance.

Adult beetles are often found in abundance about the flowers of *Nymphaea advena* and it may be that they are concerned in some way in the pollination of the flower. They were observed feeding upon the pollen and pollen is commonly dusted over the legs and body of those that crawl about over the stamens.

Reaction to Submergence

Submergence of the adults due to wave action or to the accidental submergence of the supporting leaf is not uncommon in the normal environment, but evidently is not usually accompanied by serious results. On passing under the surface of the water the beetle becomes surrounded by a film of air. Usually, after a short sojourn under water, the elytra are raised slightly and it comes to the surface, remains quiet for a short time, moves about on the surface film, and then flies to a nearby leaf. Some variation of this response occurs but the end results are essentially the same.

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DEPARTMENT OF SUMMARIES

DEVOTED TO DIGESTS OF PROGRESS IN BIOLOGY

CHECK LIST OF IMPORTANT REFERENCES DEALING WITH THE TAXONOMY OF FUNGI

By

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In the study of the fungi and especially the determination of species there is a need for a complete list of the best and recent works on mycology. Many of the present day mycologists maintain up to date files of the literature on the revisions of groups and the classifications of the fungi. Many of us having entered the profession only recently, have not been able to attempt or maintain an index of this kind. Many of us have relied upon Saccardo's "Sylloge Fungorum" but these volumes are long out of date, their publication having ceased upon or shortly before the voluminous works of von Höhnel, Diedicke, Sydow, Theissen, and others. Lindau's "Thesauraus Literaturae Mycologicae" does not fulfill the purpose of this manuscript. The first few volumes of this work arranged alphabetically according to authors, are helpful for information on completing citations. They are especially helpful in locating the publications of species of fungi whose citations have so carelessly been omitted or so incompletely published in Saccardo. The more recent volumes contain the citations of the earlier volumes, arranged according to subject matter. The citations are old and arranged under large groups so that considerable time is required for finding desired literature, at least much more than it would require were the citations placed respectively under families and these under orders, etc., as is the plan of this bibliography.

In 1918, Bessey prepared a preliminary bibliography of mycological literature. Many selected references were incorporated in this article and most of the older literature cited in his publication have been omitted in this paper. Our publication deals almost entirely with the world's literature of fungus monographs and revisions after the year 1900. For the literature prior to that date Lindau's "Thesauraus Literaturae Mycologicae" will be found useful as well as the list of Bessey. The citations incorporated in this manuscript are up to date. They include all of the important works of von Höhnel, Sydow, Theissen, Petrak, and others arranged alphabetically according to the author under their respective families in the system of the fungi. The first chapter contains citations to general works on the classification of the fungi and the remaining chapters contain the citations to publications of special groups of fungi.

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DEPARTMENT OF METHODS, REVIEWS, ABSTRACTS AND BRIEFER ARTICLES

SOME TECHNICAL METHODS FOR HYDRA AND PLANARIA

By L. H. Hyman
Department of Zoology, University of Chicago

The following notes are published in the thought that they may be of use to others. The methods are intended chiefly for the preparation of whole mounts and of preserved material.

1. Method of cultivating Hydra. In order to obtain first class specimens of Hydra for histological purposes, it is necessary to grow them in the laboratory. Laboratory reared specimens are paler and clearer than those collected from nature. The species *Hydra vulgaris* (formerly called *grisea*) is much more favorable for technical purposes than the more common *Hydra oligactis* (formerly *fusca*) owing to its lack of pigment.

For the successful cultivation of Hydra an abundant supply of food must be provided. The food animals most acceptable to Hydra are copepods, cladocerans, insect larvae, and small aquatic oligochaetes. Ostracods are generally, although not always, rejected. Insect larvae and oligochaetes, although eagerly accepted by Hydra, do not constitute a favorable food supply as they do not move about sufficiently to enable the Hydra to capture them. Cladocera, owing to their active habits and parthenogenetic mode of reproduction, have been found to constitute the best food for laboratory cultures. Although other genera will serve, the genus *Ceriodaphnia* has proved the most favorable for the purpose. It is easily cultivated, it is very active, and is of about the right size.

The following method is used to rear *Ceriodaphnia* and other small crustaceans. Vigorous sprays of water weeds, such as *Elodea* and *Ceratophyllum*, and also masses of green algae are boiled in a small quantity of water for fifteen minutes, in order to kill them. The dead plants are then placed in jars of water, using sufficient plant material in each jar to cover the bottom with a greenish layer when the plants have decayed. After a few days daphnids are put into the jars. They will be found to multiply abundantly in such cultures. A few grains of wheat (killed by boiling) or small pieces of fresh liver may be added to the jars from time to time.

At intervals a portion of the water should be removed and fresh water added. Such cultures will flourish for several weeks but eventually new cultures must be prepared and the daphnids transferred to these.

At the beginning Hydra must of course be collected from out-of-doors. The animals should be placed in clean jars of water which should have stood for a day or two in advance. The presence of green plants is not at all necessary and is often a disadvantage as the plants may grow to such an extent as to crowd the jar. Daphnids are dipped up from the culture jar and added to the jar containing the Hydra every day or two. It is also quite feasible to grow Hydra in the same culture jar with the daphnids, providing the water in the latter is clear. After a daphnid culture has begun to flourish, a few Hydra may be put into it, taking care that they do not fall to the bottom. The culture then requires no further care for some time, but usually in the end the Hydra have increased so greatly that the reproduction of the daphnids does not keep pace with them.

2. Method of killing Hydra in an expanded condition. Select from the Hydra culture, long thin individuals, avoiding those which have recently fed (unless of course specimens containing food are desired for some especial reason). Place them in a clean finger bowl or similar vessel, handling them as gently as possible. The vessel should contain only sufficient water to permit the animals to expand fully, not more than ½ inch. As many animals may be placed in the dish as space permits and they should be arranged so that they do not touch each other. It will be found that when the vessel is allowed to stand quietly the animals will expand in a short time. They should be killed at once, as after a time they are likely to contract. Heat some Bouin's fluid hot, not boiling (Bouin's fluid: 75 cc. saturated aquaeous solution of picric acid, 25 cc. undiluted formalin, 5 cc. pure glacial acetic acid). Suddenly dash the hot Bouin's fluid into the vessel containing the expanded Hydra, using two to three times as much fixative as there is water in the vessel. The temperature of the mixture in the vessel should not exceed 60°C. The animals will be found to be killed in an expanded condition. Remove at once to cold undiluted Bouin's fluid, leave for thirty minutes, and then wash in alcohol as usual. Preserve in 70% alcohol and proceed as usual for whole mounts.

3. Method of killing Planaria in a flattened, expanded condition. Planaria should not in general be fixed immediately after feeding, as the intestine will burst through the body wall. For most purposes the animals should have been starved several days. Select individuals of normal shape and proportions, avoiding those that have recently undergone fission. Place on a glass plate and remove most of the water from the

animal. As the animal is crawling with its body extended in a straight line, drop upon it two or three drops of 2% nitric acid. The animal will be found to die in an expanded, flattened, relaxed condition. It is important that the nitric acid be dropped simultaneously over the whole animal. As soon as dead, cover with two or three drops of Gilson's fluid or other fixative of which the chief constituent is corrosive sublimate. Pick up the worm with a piece of stiff paper and immerse in a dish of the fixative. Fix for thirty to sixty minutes and proceed as usual. Corrosive sublimate fixatives have been found to be the best for Planaria; fixatives containing picric acid seem unfavorable. With a little practice it is easy to kill eight to ten worms simultaneously by this method, placing the worms in a group on the glass plate.

4. Method of flattening Planaria after fixation. If the worms are still not flat enough for whole mounts, for they tend to bend when put into the fixative even when killed flat, the following method will flatten them perfectly. Wash after fixation for two hours in running water or water changed several times. Place in 35% alcohol for thirty minutes; then transfer to 50% alcohol, and at once put the worms into a dish like a Stender dish with a flat bottom. Spread them out over the bottom so that each worm is in direct contact with the bottom. Remove nearly all of the alcohol, leaving only enough to keep the worms moist. The worms will be held flat against the bottom of the vessel by surface tension. It is usually necessary to press those which are much curved gently with an instrument until they touch the bottom at all points. Leave for 24 hours. Then add a small quantity of 70% alcohol without disturbing the worms and leaving them still adherent to the bottom. Leave for 48 hrs. Keep covered tightly throughout the flattening process. Then proceed as usual. The worms will be found to be perfectly flattened. It is probably unnecessary to add that after corrosive sublimate fixation, great care must be taken to remove the sublimate.

5. Method of preparing whole mounts of Planaria to show the digestive tract. Ordinary preparations of Planaria do not show the digestive tract. By the following method this system may be caused to stand out brilliantly. Select worms of normal shape and proportions and of small size. Starve them for one to two weeks. Then feed them on blood clot. The blood clot must be clean and fresh. Ox blood clot has been chiefly used by the writer but other vertebrate blood has been found equally acceptable to the worms. The blood should be taken directly from the killed animal into a clean bottle and allowed to stand quietly until a firm clot is formed. This is cut into small pieces, washed gently with water, and the pieces placed in the dish with the worms. The animals from which the blood is taken must not of course be anaesthetized as the anaesthetic in the blood renders it unacceptable. The worms should not be disturbed during the

feeding process. Not earlier than eight hours nor later than eighteen hours after feeding the worms should be killed by the foregoing method. Any of the usual whole mount stains can be used on them; haematoxylin stains have given excellent results. Every detail of the digestive tract will appear in brilliant fashion in such preparations. The digestive tract is yellow owing to the ingested blood corpuscles in the entoderm cells; the digestive tract does not stain and hence appears in sharp contrast to the remaining stained regions of the animal.

PROCEEDINGS OF THE AMERICAN MICROSCOPICAL SOCIETY

Minutes of the Cincinnati Meeting

The forty-second annual meeting of the American Microscopical Society was held in affiliation with the American Association for the Advancement of Science at Cincinnati, Ohio, December 28, 1923.

President Chancey Juday presided at this meeting.

The report of the Treasurer for the year 1923 was read by the Secretary, accepted, and referred to an auditing committee composed of H. J. VanCleave and Frank Smith.

The report of the Custodian was read by the Secretary, accepted, and referred to an auditing committee composed of Edw. Pennock and F. E. Ives.

The meeting expressed itself as greatly gratified by the present state of the Spencer-Tolles Fund which now exceeds $10,000.00. It also voted to extend hearty congratulations to Custodian Magnus Pflaum for the splendid growth of the fund.

The Secretary presented a report on the affairs of his office.

The following officers were nominated and elected: President, Dr. B. H. Ransom, Bureau of Animal Industry, Washington, D. C.; First Vice-President, Professor F. H. Krecker, Ohio State University, Columbus, Ohio; Second Vice-President, W. E. Allen, Scripps Institution, La Jolla, California.

Professor George R. La Rue, University of Michigan, Ann Arbor, Michigan; Professor Z. P. Metcalf, North Carolina State College of Agriculture and Engineering, Raleigh, North Carolina; and Professor E. M. Gilbert, University of Wisconsin, Madison, Wisconsin were chosen as the elective members of the Executive Committee for 1924.

Adjourned.

Paul S. Welch, *Secretary.*

CUSTODIAN'S REPORT FOR THE YEAR 1923

Spencer-Tolles Fund

Balance December 1922		$ 9535.47
Interest on bonds		250.00
Dividends Stock Penna. R. R.		129.00
Dividends Stock Buildg. & Loan Ass'n		177.00
Cash from Wm. F. Henderson, Treasurer		50.00
		10141.47
Increase during the year	$ 606.00	

Totals

All contributions	802.03	
All sales of Transactions	1193.38	
All Life memberships	300.00	
All Interest, dividends, &c	8156.06	
	$10451.47	
Less all Grants	310.00	10,141.47

INVESTMENTS

Bonds, Rio Grande Junction R'y	5000.00	
Stock, Penn'a R. R.	2150.00	
Stock. Keystone B. & L. Ass'n	2991.47	10,141.47

Contributions of $50. and over: John Aspinwall, Iron City Microscopical Society, Magnus Pflaum, Troy Scientific Society.

M. PFLAUM
Custodian

Phila., Pa., Jan. 11, 1924.

Having examined the above account, and the securities on hand as shown therein, we find them correct.

F. E. IVES
EDWARD PENNOCK
Auditing Committee

ANNUAL REPORT OF THE TREASURER OF THE AMERICAN MICROSCOPICAL SOCIETY

December 13, 1922 to December 10, 1923

RECEIPTS

Balance on hand, December 13, 1922	$ 750.48
Dues received for Volume 41 or before	60.00
Dues received for Volume 42	250.11
Dues received for Volume 43	250.07
Dues received for Volume 44	9.50
Initiation Fees	90.00
Subscriptions for Volume 41 or before	9.00
Subscriptions for Volume 42	272.98
Subscriptions for Volume 43	77.87
Subscriptions for Volume 44	3.00
Sales of Transactions, duplicates, back numbers	107.35
Advertising, Volume 40, or before	85.00
Advertising, Volume 41	297.50
Advertising, Volume 42	5.00
For plate of Mary A. Booth	6.45
Interest on bank deposits	4.82
Total	$2279.13

EXPENDITURES

Printing Transactions, Volume 41, No. 4	$ 171.53
Printing Transactions, Volume 42, No. 1	412.11
Printing Transactions, Volume 42, No. 2	282.05
Printing Transactions, Volume 42, No. 3	130.72
Postage and express for Secretary	49.85
Postage and Express for Treasurer	15.00
Office expenses of Secretary	63.69
Office expenses of Treasurer	29.40
Office expenses of Custodian	5.00
Secretary, trip to Boston	50.00

C. S. Schultz, purchase of 1 set Transactions	37.00
Spencer-Tolles Fund, payment of loan	50.00
Balance on hand	982.78
Total	$2279.13

December 10, 1923.

W. F. HENDERSON, *Treasurer.*

We have examined the accounts of Mr. W. F. Henderson, Treasurer of the American Microscopical Society, for the period beginning December 13, 1922 and ending December 10, 1923. We have checked the entries of revenues and expenditures and find that the reported balance of these accounts agrees with the bank balance as shown by the Pass Book of the Society.

H. J. VAN CLEAVE
FRANK SMITH
Auditing committee.

January 8, 1924.

REPORT OF THE SPENCER-TOLLES FUND COMMITTEE

The American Microscopical Society has great reason to be gratified with the progress that has been made in bringing the Spencer-Tolles Fund up to a point for which we have long been striving. The total of the Fund has now passed $10,000. That goal set by our worthy and able Custodian some years ago has been achieved only through his indefatigable labors in guarding the funds and the Society owes him much for his unstinted services in this direction.

It appears as if the principal were now large enough to justify devoting the income more distinctly to the encouragement of research than has hitherto been done and less exclusively to providing for further accumulation. We do not mean to indicate that the Society should decide to spend the income whether or no; and in order to make this position clear it may be necessary to refer to the announced policy of the Committee as expressed in publications as well as in correspondence with applicants who ask consideration for grants from the Fund. In the past the Committee has constantly refrained from recommending the appropriation of any more than occasional minor grants. It has recommended that the major part of the income be added to the principal and invested by the Custodian so that the Society might be able to accumulate a fund sufficient to attract attention and serve more worthily the purposes for which it was originally intended. Now that the Fund has reached the goal we have long sought it certainly will be possible to devote $250 a year, or in some exceptional case more, but with an absolute limit of $500 in any one year *provided* the applications seem to justify such expenditure. The Committee and the Society is certainly charged with the responsibility of seeing that applications for grants are carefully studied and recommendations made or approved only when the circumstances clearly justify the investment. It seems wise to lay this matter of general policy before the Society in order that it may be discussed and eventually determined by the organization rather than by the Spencer-Tolles Fund Committee.

The Committee has now before it some specific applications which undoubtedly will serve as a basis for formulating requests and making grants of an appropriate character that will involve larger sums than hitherto given. We believe that in accordance with previous practice the Society does not desire to discuss details concerning grants, but wishes the Committee to make a careful study of each situation and to report its findings. To avoid misunderstanding it should be distinctly understood that the Committee welcomes at all times opportunity to lay the fullest information desired before the Society or any part of it.

Members should not fail to keep in mind the advantages of still further increasing the Fund by personal gifts or solicitation from others. Nothing is more lasting in its benefits than a fund which contributes to the advancement of knowledge. Will you not aid? Contributions or suggestions may be sent any member of the Committee and will be gladly received.

(signed)

HENRY B. WARD (Chairman)
SIMON H. GAGE
N. A. COBB.

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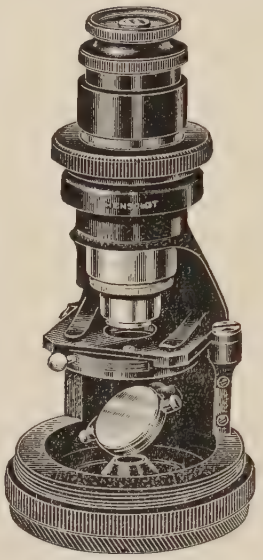

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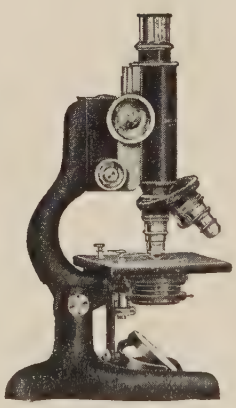

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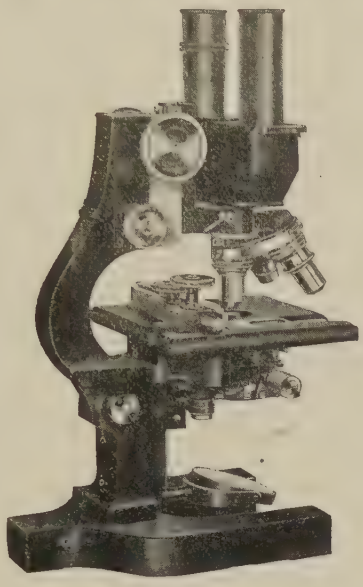

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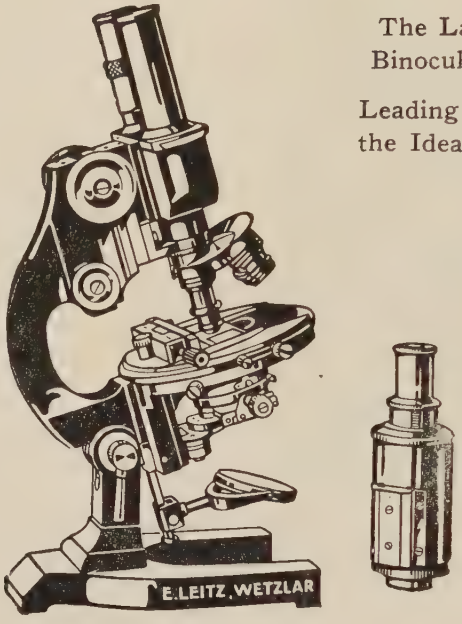

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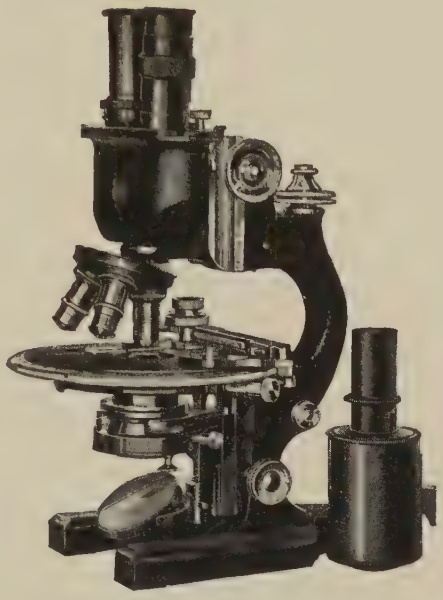

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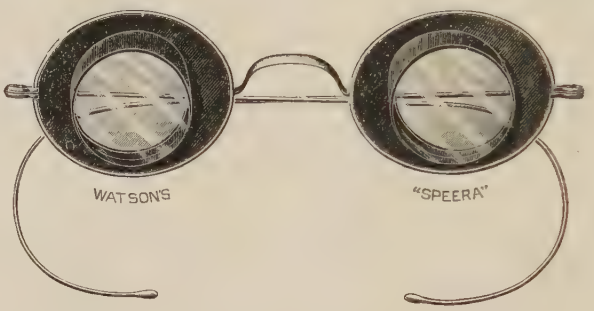

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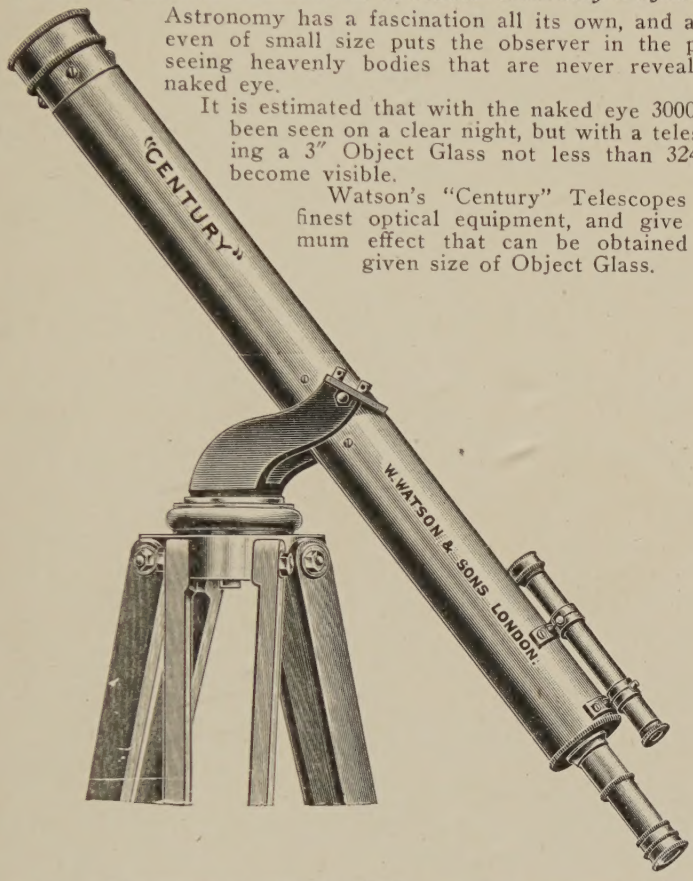

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Correspondence relative to membership, publication, exchanges, advertising, etc., should be addressed to the Secretary-Editor.

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